Supplementary Information (SI) for Analytical Methods.
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Supporting Information for

Multidimensional information detection of liver cancer cell exosomes treated with Shikonin

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S1. Experimental and method

S1.1 Cells and Reagents

The human hepatoma cell line SMMC-7721 cells, HepG2 cells and human cell line HL-7702 cells were purchased from the Cell Bank (Shanghai, China). SMMC-7721 cells, HepG2 cells and HL-7702 cells were cultured under standard culture conditions with 37°C and 5% carbon dioxide. The SMMC-7721 cells, HepG2 cells and HL-7702 cells were incubated in the RPMI-1640 medium (GIBCO, USA) with 10% FBS and 1% penicillin/streptomycin (Invitrogen). The Phosphate Buffered Saline (PBS) was used to rinse the petri dishes two or three times after removing the supernatant when the cells grew to 80% in the petri dishes. Then, the RPMI-1640 medium containing 10% exocrine serum was added to the petri dishes to remove the effect of exosomes contained in FBS. The supernatants of SMMC-7721 cells, HepG2 cells and HL-7702 cells were collected respectively after 48 hours.

S1.2 Exosomes extracted by ultrafiltration

The supernatants of SMMC-7721 cells, HepG2 cells and HL-7702 cells were collected, respectively, and subjected to differential centrifugation: 2000 g for 10 min and then 10 000 g for 15 min to remove the cell debris. The supernatant obtained by centrifugation was filtered with 0.22 μm filters (Millipore) and then added into Amicon®ultra-15 ultrafiltration tubes (Aperture: 100 kDa), centrifuged at 4000 g for 30 min, and emptied into collection tubes. Finally, PBS was added as a replacement. The sample was gently aspirated several times and centrifuged at 4000 g for 0.5 h. The treated SMMC-7721-exos, HepG2-exos, and HL-7702-exos were aspirated from the

concentration tubes, subsequently resuspended in PBS, and aliquoted for further use. The SMMC-7721-exos, HepG2-exos and HL-7702-exos could be immediately used for subsequent experiments or stored at -80 °C.

S1.3 Scanning Electron Microscope (SEM)

The morphologies of exosomes and cells were investigated by SEM. Briefly, 1 μl of exosomes was diluted 1000 times with PBS. A drop of 10 μl was put on the mica surface and dried at 25 °C. About 1×10^4 cells were plated onto coverslips. The cells were rinsed with PBS after 24 h, followed by fixation with 2.5% glutaraldehyde for further 12 h at 4 °C. Later, the coverslips were rewashed with PBS, followed by graded ethanol. The specimens were then coated with Au (5 nm) before SEM observation (FEI Quanta 250 FEG).

S1.4 Western blotting (WB)

Purified SMMC-7721-exos, HepG2-exos and HL-7702-exos used for WB were extracted with RIPA buffer (high) (Solarbio, China) containing protein phosphatase inhibitors (Solarbio, China). The proteins were quantified using the BCA™ Protein Assay (Solarbio, China). 10% SDS-PAGE was employed to separate the protein lysate, which was then electrophoretically transferred to a nitrocellulose (NC) membrane. A 5% skimmed milk powder solution was used to seal the NC membrane. The primary antibodies of CD9 and TSG101 were incubated with the membrane at 4 °C overnight. The secondary antibodies were added and incubated after washing. Finally, the ECL luminescent solution (Millipore) was dripped evenly on the imprinted membrane and incubated at 25 °C for 1-2 min. The Chemoluminescence Imaging System (Analytik

Jena, Germany) was used to capture the chemiluminescence signal and analyze the intensity quantitatively. Each assay was repeated thrice. A representative image of three independent experiments was shown. Another group of WB results of SMMC-7721-exos, HepG2-exos, and HL-7702-exos was shown in Fig.S1. WB results of Calnexin as a negative marker were shown in Fig.S2.

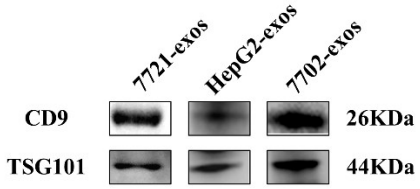

Fig. S1 Another group of WB results of SMMC-7721-exos, HepG2-exos, and HL-7702-exos.

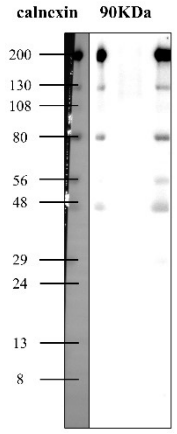

Fig. S2 WB results of Calnexin as a negative marker.

S1.5 Nanoparticle Tracking Analysis (NTA)

The size distribution and particle concentration of purified exosomes were analyzed using a PMX120 system (PARTICLE METRIX, Germany) equipped with particle-tracking software and a red (642 nm) laser. Briefly, exosomes isolated from each cell line were diluted by 100- to 1000-fold with PBS to the appropriate concentration for the analysis, a range from 1 to 10 $\times 10^8$ particles/ml. All acquisition settings, including capture time, camera level, and detection threshold, were kept the

same in all samples to minimize variability. For each sample, the measurements were performed in triplicate under identical analysis settings.